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In Vivo Selection for Transmissible Drug Resistance in *Salmonella typhi* during Antimicrobial Therapy

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We report the recovery of *Salmonella typhi* that acquired resistance to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and gentamicin subsequent to multiple antibiotic therapy. *Escherichia coli* and *Klebsiella pneumoniae* isolates which were recovered from the same stool sample displayed identical resistance patterns. Agarose gel electrophoresis revealed that *S. typhi* and laboratory-derived transconjugants contained a high-molecular-weight plasmid present in the resistant intestinal bacteria.

Typhoid fever caused by *Salmonella typhi* continues to be a common endemic illness in developing countries. In the United States the incidence has decreased, although sporadic disease still occurs (4). Travel to an endemic region and poor sanitary conditions are factors which contribute to outbreaks (4). In contrast to most enteric pathogens and other *Salmonella* species, *S. typhi* generally remains susceptible to prescribed antibiotic therapy. Chloramphenicol is the antibiotic of choice, particularly in severely ill patients (4, 21). Ampicillin and trimethoprim-sulfamethoxazole are also effective agents (12, 23). Resistance to these antimicrobial agents is usually mediated by R-plasmid transfer. Outbreaks of infection with chloramphenicol-resistant *S. typhi* in Mexico, Peru, Vietnam, and elsewhere have been documented (3, 8, 15, 19), and ampicillin resistance and, less frequently, trimethoprim-sulfamethoxazole resistance have also been reported (18, 26). In addition, a combination of resistance to two of these agents has been reported (5–7); however, recovery of *S. typhi* resistant to all three antibiotics is quite rare (7, 14), and there are no reports of such an isolate in the United States. The in vivo acquisition of antibiotic resistance in *S. typhi* from a 25-year-old male in North Carolina being treated for typhoid fever and having no history of foreign travel is reported here.

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Two blood cultures obtained from our patient on admission were positive for *S. typhi* (13) which was susceptible to all antibiotics (*S. typhi* S). The patient's hospital course was complicated by an antibiotic (ampicillin)-induced urticarial rash, and he was sequentially treated with ampicillin (days 1 to 3), chloramphenicol (day 4), and trimethoprim-sulfamethoxazole (days 5 to 12) prior to resolution of disease. Broth macrodilution MICs (11) demonstrated that *S. typhi* recovered from stool 16 days after the initiation of antibiotic therapy (*S. typhi* R) had acquired high-level resistance to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole (MICs of >128, >128, and >6.75/>128 μg/ml, respectively) as well as to gentamicin (MIC of 64 μg/ml). Susceptibilities to other antibiotics, as determined by disk diffusion (1), were as follows: susceptible—amikacin, cefoxitin, ceftriaxone, cefuroxime, ciprofloxacin, imipenem, norfloxacin, and tetracycline; intermediate—amoxicillin-clavulanic acid (Augmentin) and cefazolin; resistant—cefoperazone, kanamycin, mezlocillin, piperacillin, ticarcillin, and tobramycin. *S. typhi* R also tested positive for production of β-lactamase. The *S. typhi* blood and stool isolates were identical biochemically and were of the same phage type, E1 (courtesy of J. J. Farmer III and F. W. Hickman-Brenner, Enteric Bacteriology Section, Centers for Disease Control, Atlanta, Ga. [10]). MICs of ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and gentamicin for two enteric commensal organisms, *Escherichia coli* and *Klebsiella pneumoniae*, which were recovered from the same stool sample were nearly identical (within a twofold dilution) to those for *S. typhi* R. Disk diffusion susceptibilities to other tested antibiotics were also very similar, with the exception that *E. coli* showed resistance to cefoxitin and amoxicillin-clavulanic acid.

S. typhi R was passed nonselectively (via a daily "sweep") for 2 weeks on 5% sheep blood agar containing no antibiotics. Fifty CFU were then individually tested for antimicrobial susceptibility by the disk diffusion procedure. All clones remained resistant to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and gentamicin, indicating that the resistance phenotype was stably maintained.

A filter mating conjugation procedure was performed to determine whether resistant stool isolates could transfer antibiotic resistance to the *S. typhi* S blood isolate (17). Rifampin-resistant *S. typhi* S (selected on plates containing 50 μg of rifampin per ml) was added to one of the presumed donor enteric strains or *S. typhi* R at a 10:1 ratio (10^7 *S. typhi* and 10^6 enteric CFU/200 μl) and pipetted onto a 25-mm-diameter nitrocellulose membrane filter that had been placed on a MacConkey agar plate. The filter was incubated at 37°C for 2 h. Bacteria were removed from the filter by vortexing in a tube of sterile saline and cultured on MacConkey plates containing rifampin (50 μg/ml) plus either ampicillin (50 μg/ml), trimethoprim (2.5 μg/ml), sulfamethoxazole (50 μg/ml), or gentamicin (25 μg/ml). *E. coli*, *K. pneumoniae*, and *S. typhi* R were all conjugally proficient; antibiotic resistance

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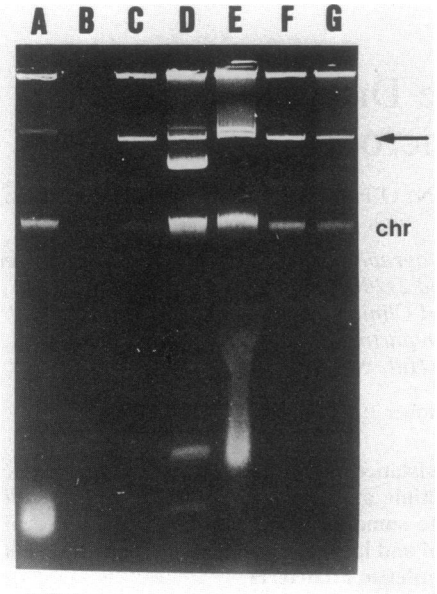

FIG. 1. Agarose gel electrophoresis of plasmid DNA prepared from clinical isolates and *S. typhi* S transconjugants. Plasmids were separated on a 0.7% agarose gel and stained with ethidium bromide prior to photography. Lanes: A, R478 marker plasmid (165 megadaltons); B, *S. typhi* S; C, *S. typhi* R; D, *E. coli* stool isolate; E, *K. pneumoniae* stool isolate; F, *S. typhi* S transconjugate JPS101 (*E. coli* donor); G, *S. typhi* S transconjugate JPS104 (*K. pneumoniae* donor). Acquisition of a single high-molecular-weight plasmid is indicated by the arrow. chr, Chromosomal DNA.

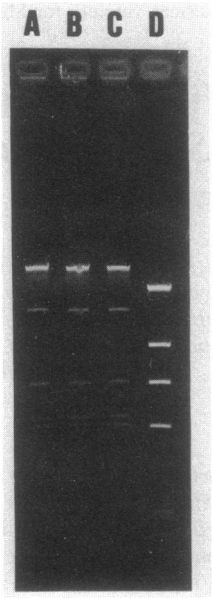

FIG. 2. *Bam*HI digests of plasmid DNA from *S. typhi* R (lane A), JPS101 (lane B), and JPS104 (lane C). Lane D is *Hin*dIII-digested lambda DNA marker fragments.

was transferred to *S. typhi* S at high frequencies ranging from 1.4×10^{-2} to 1.2×10^{-1}. The transfer frequency of antibiotic markers was not dependent on the selective agent. More than 95% of the *S. typhi* S transconjugants were resistant to all selected antibiotics, suggesting that genes coding for antibiotic resistance determinants were closely linked. Resistance markers were transferred in toto to *E. coli* K-12 (kind gift of Ken Bott, University of North Carolina, Chapel Hill) at a frequency of approximately 7×10^{-3}. Similarly, *E. coli* K-12 (containing the R factors from *K. pneumoniae*) was able to transfer resistance to clinical isolates of *K. pneumoniae*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* at a frequency of approximately 3×10^{-3}.

Plasmid DNA was extracted from the clinical isolates and laboratory-derived resistant transconjugants by a rapid alkaline lysis technique as described by Birnboim and Doly (2). Electrophoresis on a 0.7% agarose gel and ethidium bromide staining of plasmid DNA was performed by the procedure of Maniatis et al. (16). Figure 1 demonstrates acquisition of a single high-molecular-weight plasmid migrating slightly faster than the 165-megadalton marker plasmid (kind gift of D. Taylor, University of Alberta, Edmonton, Alberta, Canada [27]) that was associated with resistance to ampicillin, chloramphenicol, trimethoprim, sulfamethoxazole, and gentamicin. The plasmid appeared to have similar molecular weights in *S. typhi* R, enteric stool isolates, and *S. typhi* S transconjugants JPS101 (*E. coli* donor) and JPS104 (*K. pneumoniae* donor). *Bam*HI restriction enzyme digests of plasmid DNA purified from strains *S. typhi* S, JPS101, and JPS104 (Fig. 2) confirmed that the antibiotic resistance genes were located on a unique plasmid. Identical restriction endonuclease patterns were also seen when plasmid DNAs from these strains were digested with *Eco*RI or *Hin*dIII (data not shown).

The *S. typhi* isolates recovered from this patient represent a first reported incidence in the United States of in vivo acquisition of resistance to all standard first-line antibiotics for the treatment of typhoid fever. His treatment course consisted of consecutive administrations of ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole, suggesting that these antibiotics played a selective role for the emergence of a resistant organism. Fortunately, our patient had clinically improved when *S. typhi* R was recovered from his stool sample, and the organism was not present upon two subsequent cultures. It was unknown how this patient initially acquired *S. typhi*. He had no identifiable risk factors such as foreign travel, drinking from a well source, or contact with diseased individuals.

Accounting for the presence of commensal intestinal flora which were multiply antibiotic resistant was also uncertain. Presumably, these organisms also emerged under selective antibiotic pressure and were the source of transmission of resistance to *S. typhi*. Nosocomial transmission of resistance markers was unlikely, since this novel resistance pattern was not seen in members of the family *Enterobacteriaceae* isolated from North Carolina Memorial Hospital during the time of his hospital stay.

All antibiotic resistance determinants appeared to reside on a single large plasmid which was self-transmissible with a wide host range. Acquisition of drug resistance in vivo by *S. typhi* has been reported previously (5, 6, 14, 22); however, there is only one report of a strain becoming resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole (14). The isolate we describe differed, because lactose fermentation was not cotransmissible with drug resistance (our strain did not ferment lactose) and resistance was stably maintained. Additionally, all resistance determinants were conjugally transferred at a high frequency. This plasmid did not seem to belong to the HI1 incompatibility group (IncHI1), which is most often associated with chloramphenicol resistance in *S. typhi* (20, 25). Like others in this group,

the R plasmid was large (greater than 100 megadaltons). However, the frequency of transfer of drug markers in IncHI1 is temperature susceptible (usually less than 10^{-5} at 37°C), and most markers mediate resistance to tetracycline (24, 25). Moreover, this plasmid was compatible with the prototype IncHI1 plasmid, R27 (9).

This report demonstrated that successive treatment regimens with different antimicrobial agents resulted in selection of an *S. typhi* strain resistant to all drugs commonly used for typhoid fever. Transfer of resistance markers was reproduced in vitro by using donor enteric flora recovered from the same stool sample. Antibiotic therapy should be carefully monitored when treating typhoid fever. Physicians and clinical microbiology laboratories should recognize the potential for emergence of multiply resistant *S. typhi*.

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